See also: **II/Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry; Pyrolysis Gas Chromatography. **Extraction:** Supercritical Fluid Extraction.

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Liquid Chromatography

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Introduction

The goals in the use of liquid chromatography for the separation of polymers and polymer oligomers include the determination of purity, the production of pure/purer polymer mixtures and for obtaining quality control data for polymer intermediates. There are fundamentally two partition mechanism options for such separations: size exclusion or sorption in the sense of surface adsorption or dissolution into a stationary phase. Size exclusion involves the partition of the molecules of interest from the mobile phase into the stationary mobile phase contained in the various pores of the solid support. The extent to which the stationary liquid is explored by the polymer molecules is determined by their Stokes' radius (dynamic size) and the volume of mobile phase in pores of a diameter large enough for penetration to be possible. Adsorption onto or sorption into a phase coated or grafted as a thin film on the surface of a solid support is dominated by solubility in the mobile phase and the chemical potential for sorption of the polymer molecules in a given mobile phase in contact with a given stationary phase. Because stationary phase supports used in modern liquid chromatography are themselves porous, mixtures of size exclusion in the presence of sorption and *vice versa* are known.

Size Exclusion

Historically, size exclusion has been the method most often used for polymer separation, purification and molecular weight determinations. The technique developed in parallel in the 'organic' polymer area and the biological polymer area. When used in organic polymer work, the term 'gel permeation' is used. In water soluble biopolymer work, it is called 'gel filtration'. There is no fundamental difference in the principles involved and both are size exclusion based. In liquid chromatography molecules move in the direction of development because of mobile phase flow. Most gel electrophoresis methods are, in reality, size exclusion based separations and that includes the gel methods used for sequencing of nucleotide fragments. There the driving force is electromigration of molecules with essentially identical ionic mobility which reptate through a porous polymer medium at rates proportional to size.

Sorption

Despite the potential attractiveness of a method which could introduce chemical selectivity in to the separation of polymers, sorption methods have seen little practical application until more recent times. The sole exception is the ion exchange purification of polyelectrolytes such as proteins. The history of the development of polymer high-performance liquid chromatography (HPLC) is an interesting one and is detailed in subsequent paragraphs. The reader should keep the following introduction in mind when

considering various models for retention of polymers in sorption techniques.

The liquid chromatography of small molecules is dominated by solubility of the molecule[s] of interest in the moving or mobile phase. This is in stark contrast to gas chromatography where the stationary phase contribution dominates. One controls retention and the large fraction of selectivity (differential migration) in liquid chromatography by mixing various solvents to obtain differential solubility sufficient for the task of separation. That is not to suggest that the stationary phase is not important. It is, but generally as a secondary effect.

The rate of change of retention volume (or time at constant flow) for small molecules (m.w. < 2 kD) is not steep compared to polymers. Thus the strategy for small molecule separations is to use combinations of solvents in which one solvent is a good solvent and the other is more hostile or 'poorer' in terms of solubility of the molecules of interest. Polymers, on the other hand can have very rapid transitions from soluble to insoluble over narrow ranges of good/poor solvent mole fraction.

It is also common practice to inject samples of small molecules in a solvent which is good compared to the mobile phase. This technique can have awkward effects in polymer chromatography. If the mobile phase is one in which the solubility is already very small or near zero, the injected plug of good solvent moves with the leading and trailing edge of the plug being depleted of solute. The net effect is to coat the column with polymer with the excess eluting at the void volume. Columns are well-designed packed beds and, as such, are very poor mixers. The solution is to dissolve the polymer in the mobile phase and to mix the sample solution with the mobile phase before column contact.

There has been much interest in methods for the fractionation of macromolecules using reversed-phase, high-performance liquid chromatography (RPLC). Reversed-phase methods are those that have a relatively polar mobile phase and relatively non-polar stationary phase, e.g. water and paraffin oil. If it were possible to achieve both isocratic and gradient elution of polymer oligomers and isomers, then these separation techniques could provide vastly more insight and control than is currently the case in a majority of the applications where size exclusion alone dominates.

Certainly debates over the validity of models are an important scholarly aspect of the current dialogue. Successful models based on sound physico-chemical principles could guide the development of practical applications. The short-term solution is likely to be a combination of models if the historical case for the application of HPLC to small molecules applies here.

Although there are many reported successful examles of polymer separations and several models have been suggested, the retention mechanism of polymers in RPLC remains unclear. Glöckner suggests a 'precipitation–redissolution' model for the gradient elution of polymers. In this model, polymer molecules repeatedly precipitate onto the stationary phase and redissolve into the mobile phase until finally eluting at a mobile phase composition at which the polymer is totally soluble. Retention depends solely on the mobile phase with the column playing a passive role providing only a large surface area as support for the precipitate. Armstrong, Martire, Boehm and Bui proposed a model for critical solvent composition behaviour found by some in the isocratic elution of polymers. This model is often called BMAB theory or critical solution theory. This theory was developed from a statistical treatment of the equilibrium distribution of infinitely dilute polymer molecules between a mobile phase and a stationary phase based on the Flory–Huggins theory. According to the model, the range of the mobile phase composition within which finite retention factor (k) values can be observed under isocratic elution conditions is very narrow for high molecular weight polymers. Plots of log k versus the mobile phase composition show slopes that mean that polymer molecules are either infinitely retained or not retained at all. Therefore, isocratic retention should be impossible. It can be concluded from this model that the separation is strictly mobile-phase controlled and has little to do with the column length. If either of these models are correct in every detail, isocratic elution is impossible because, under isocratic elution, the polymer molecules either flow through the column without any retention or strongly adhere to the stationary phase without ever eluting.

In contrast, Snyder and coworkers assert that no special model is needed for the polymer retention and the traditional models can be used in interpreting the retention behaviour of polymers.

After numerous failures in attempts to reproduce the published work of others, Lochmüller and McGranaghan were the first to consider the likely fate of the injected polymer sample in the mobile phase prior to its contact with the column. They found that traditional retention behaviour was obtained only when the sample was adequately mixed with the mobile phase using a low dispersion, crocheted capillary tube placed between the sampling device and the column. They reported that polystyrenes of molecular weight ranging from 2000–2 800 000 Da could be separated under isocratic elution conditions with binary mobile phases of tetrahydrofuran/H_2O and dichloromethane/acetonitrile. Finite, non-zero k values

and linear relationships of log k versus the volume percentage of tetrahydrofuran and dichloromethane were observed. Alhedai, Boehm and Matire subsequently reported the isocratic elution behaviour of polystyrene homopolymers.

In polymer RPLC, separations are achieved by using a mixture of 'good' and 'poor' solvents as the mobile phase. A good solvent is one that is thermodynamically favourable for polymer solution and a poor solvent is thermodynamically unfavourable. Since polymers have low solubility in poor solvents, polymer samples are often dissolved in a good solvent for chromatography. The result is that the injected sample plug is a better solvent than the actual mobile phase. For hydrophobic polymers, the good solvent usually is a strong solvent in RPLC. Often sample preparation is followed by injection without any further treatment of the sample. Because chromatographic columns are, by their very design, poor mixing devices and the equilibration between the polymer sample and the mobile phase may be slow on the chromatographic time scale, the polymer sample can remain well solvated from its interior in the injection solvent and isolated from the mobile phase and stationary phase effects. Under the worst conditions, the mobile phase can be so hostile to the polymer that the polymer sample will co-elute with the injected plug of the good solvent. This 'solvent effect' could explain why normal chromatographic retention behaviour was not observed in some of the previous studies. In addition, polymer molecules can undergo various conformational changes in the chromatographic process due to the difference between the injection solvent and the mobile phase. These conformational changes can complicate separations and make reproducible, meaningful results difficult to obtain. The use of a pre-column mixer and an initial binary solvent whose composition is close to the mobile phase composition affords equilibration between the polymer and the mobile phase and also affords a more uniform elution condition.

There are, of course, significant differences in the properties that distinguish different polymer types from each other that must be taken into account in guiding method development. In the special case of some biological polymers such as proteins and polypeptides, a contribution of size exclusion and ion exchange is possible and careful manipulation of the ion exchange effect can provide resolution of genetically different isoenzymes. In other cases where the polymer has groups with little acid–base difference, as in the case in gene sequencing, it is possible to cleave the macromolecule at specific sequence events and to tag these fragments. The resulting fragments are then separated through size exclusion in a gel matrix using electrokinetic driving force through the gel space. Current applications of HPLC are more limited and most of the literature examples of protein resolution are carried out with molecules differing by thousands of Daltons and are limited to 'analytical' purposes through the use of denaturing conditions where narrow peak shape is more important than retention of native structure.

Many, if not most, common organic polymers are either non-electrolytes or strong polyelectrolytes. In the latter case, the most common situation is one in which the ionized or ionizable function is the same for each repeating unit and/or the groups have nearly identical pK_a values. Ion exchange is, thus of little use in the resolution of oligomers. In the case of non-ionizable polymers, such as the polystyrenes, the methacrylates, acrylates, polyesters and ethers, the use of RPLC offers the possibility of selectivity by both solvent effect and stationary phase interaction. If ordinary chromatography is possible, then it could be possible to resolve oligomers, to resolve copolymer variations by chemical selectivity and thus improve the quantitation as well as the isolation of such materials.

There are two solutions to the steep gradient in d ln k/d Vol%. The first is to find a second at a solvent less hostile to the polymer under investigation. The second is provided by modern instrumentation and that is that a gradient mixer will mix mixtures. This second option involves running a gradient where the A solvent or poor solvent is already a mixture and the same for B. In principle, a gradient can be run from 60% A to 58% A in a way reproducible to 1% by volume A in B. In this manner, the steep gradient in d ln k'/d Vol% is slowly traversed in the gradient mixing process. The results can be dramatic and it is possible to resolve individual polystyrene oligomers at an average molecular weight of 100 kD.

Figure 1 shows a separation of two polymethylmethacylate samples, one a synthetic sample containing lower molecular weight oligomers after gel permeation separation, and the other a nominally monodisperse 75 kD standard. **Figure 2** is the linear dependence of retention as a function of tetrahydrofuran vol% in water for a wide range of polyethylene glycol monodisperse standards. Note how the slope steepens with increasing molecular weight. **Figure 3** is a similar plot for poly-L-, poly-D- and poly-D,L-tryptophans. Note the linearity of response but also that the lines for all poly-L and the poly-D,L are not parallel despite the molecular weight being the same ($\sim$5.5 kD).

There are many good reasons to want to use a single component, isocratic elution method. The first is the cost per sample run. The second is that

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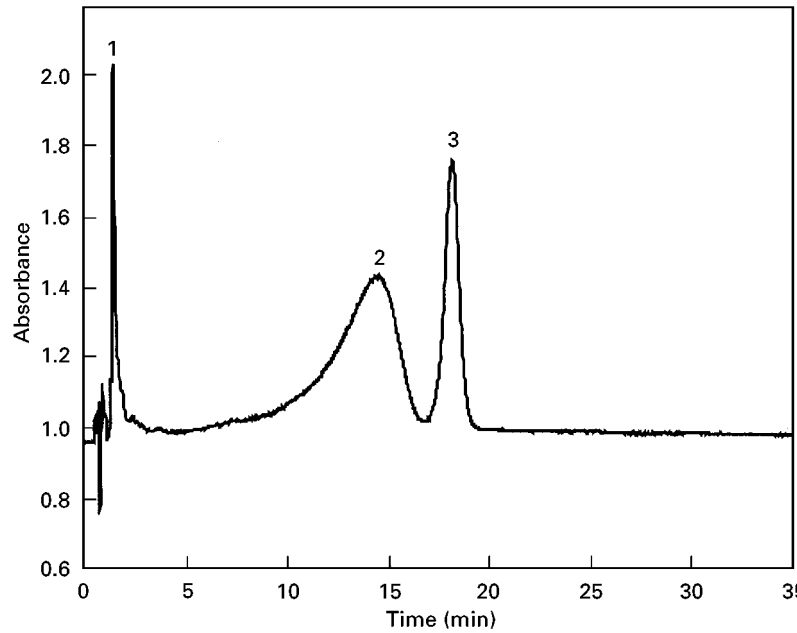


Figure 1 Separation of a mixture of PMMA 33.5-kD (2) and PMMA 75-kD (3) in gradient mode with mobile phase THF/water from 68/32 to 80/20 in 20 min on a Hypersil® 20 cm × 2 mm C18 column. (Reproduced, in part, from the author's work with permission of the *Journal of Chromatography Science* and Preston Publications.)

only one solvent need be removed from the collected polymer sample. The potential of modern liquid chromatography is to produce samples which are truly monodisperse. The fewer separation solvents the better. A possible route is the use of a single solvent and the temperature dependence of k. Of course, the magnitude of the temperature effect on retention is in direct proportion to the solubility dependence. The temperature gradient can be applied as it is in gas chromatography, i.e. as a gradient over time. Another is a gradient over space, the column length. The first example of a spatial gradient was in the separation of polyethylene glycols. These polyethers have a decreasing solubility in water with increasing temperature. Thus a column kept at high temperature will show large retention volumes for polyethylene glycols and a gradient run from hot to cold is an option. A column kept relatively hot at the inlet and cold at the outlet will show a reduced rate of development for polyethylene glycols at the

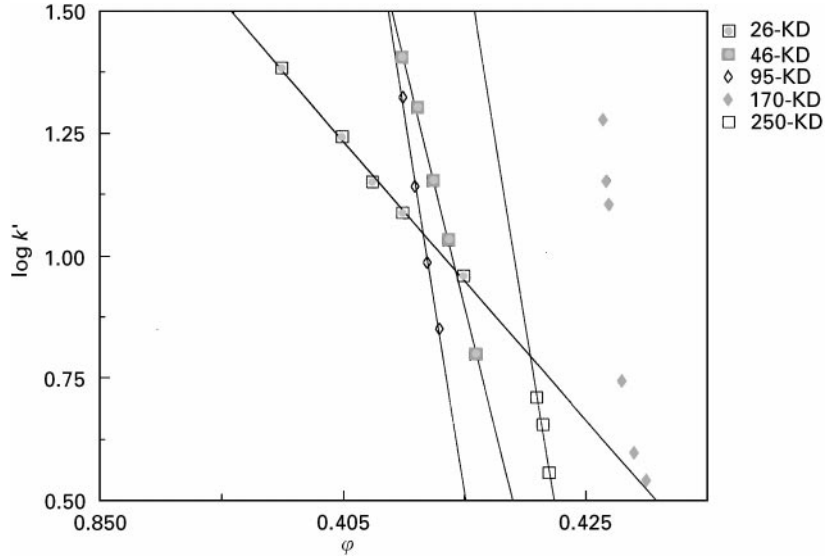


Figure 2 Linear plots of log k' (retention factor) vs. volume fraction of THF in water for PEG samples. (Reproduced, in part, from the author's work with permission of the *Journal of Chromatography Science* and Preston Publications.)

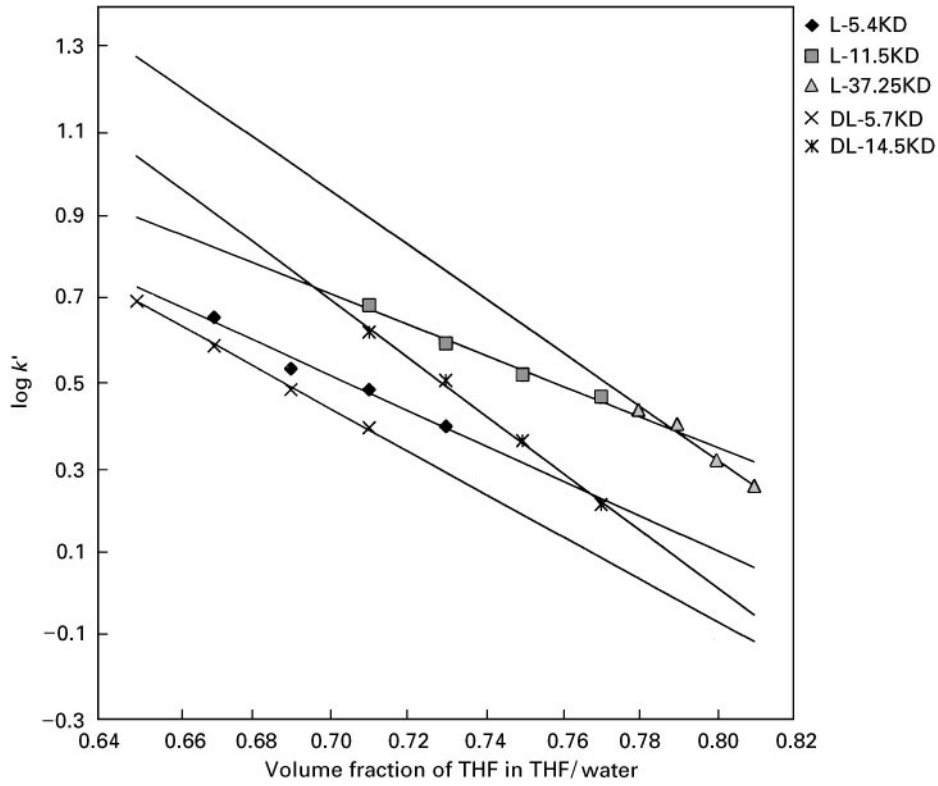


Figure 3 Dependence of log k' versus volume fraction of THF poly(L-tryptophan)s and poly (DL-tryptophan)s. (Data taken in part from work reported in Lochmüller and Chun Jiang (1994) *Journal of Liquid Chromatography* 17: 3179–3189.)

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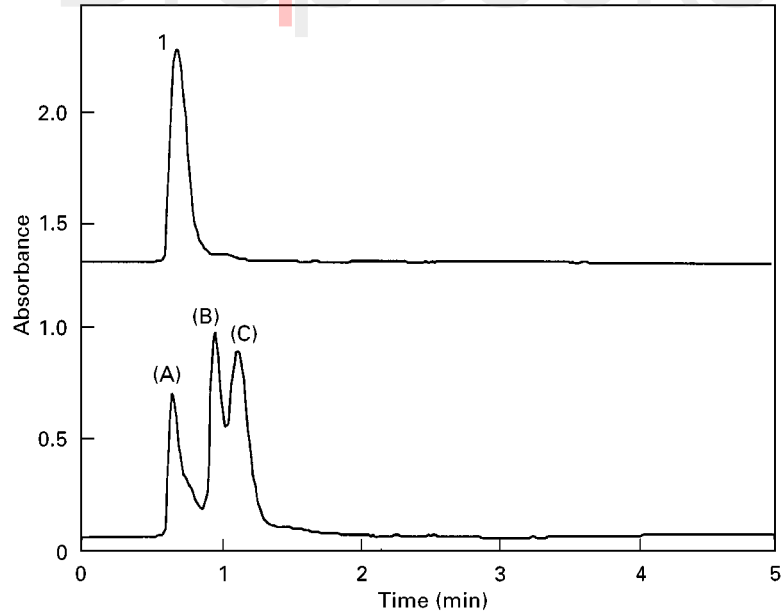


Figure 4 Chromatograms of mixture (1) of PEG 26-KD (A), 46-KD (B) and 95-KD (C) (ACN/H_2O 42/58; Top, $t = 23^{\circ}C$; the thermal gradient was $-0.05^{\circ}C\,min^{-1}$ started from 28°C. Bottom, $t_{inlet} = 40^{\circ}C$, $t_{outlet} = 23^{\circ}C$, the gradient was $0.7^{\circ}C\,cm^{-1}$ along the 10 cm column. (Reproduced, in part, from the author's work with permission of the *Journal of Chromatography Science* and Preston Publications.)

inlet and higher at the outlet. The effect is a function of molecular size within the polymer class. **Figure 4** is an example of both approaches.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases; Mechanisms: Size Exclusion Chromatography; **III/Gradient Polymer Chromatography: Liquid Chromatography. Peptides and Proteins:** Liquid Chromatography.

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Thin-Layer (Planar) Chromatography

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The use of thin-layer chromatography (TLC) in polymer analysis was first mentioned in 1968. Belenkii and Inagaki with their co-workers described separations according to the composition of random styrene–methyl methacrylate and styrene–methyl acrylate copolymers with molecular weights varying from 40 to 200 kDa. Since then polymer TLC has been developed intensively and other researchers have also begun to work actively in this field.

The mechanisms of polymer TLC have been investigated and the methods for the determination of molecular weight (MW) and molecular weight distribution (MWD) of homopolymers, such as polystyrene (PS), poly(ethylene oxide) (PEO) and poly(methyl methacrylate) (PMMA), have been developed. Impressive results were obtained for separations in accordance with different features of the polymer architecture. This investigation of structural heterogeneity of styrene–methyl methacrylate (S-MMA) copolymers have made it possible to separate random, block and alternating copolymers as well as two- and three-block copolymers. The stereoregular heterogeneity of PMMA and polybutadiene (PB) has also been determined. Styrene (S) and butadiene (BD) block copolymers have been studied and deuterated and hydrogenous PS separated.

In the vast majority of studies silica gel has been employed as a sorbent with the occasional use of alumina. In 1976 Belenkii and co-workers reported that critical conditions exist on passing from size-exclusion to adsorption chromatography of polymers. The first reviews on polymer TLC appeared in 1977. In 1980 Glöckner demonstrated the importance of gradient elution for polymer separations, and in 1982 Armstrong and co-workers used reversed-phase plates to separate homopolymers according to MW. More recent reviews have been by Glöckner (1987) with the most comprehensive review presented by Gankina and Belenkii in 1991.

Here the behaviour of macromolecules and small molecules are compared and the mechanism of chromatographic processes and the analysis of different types of polymer heterogeneity considered.

Behaviour of Macromolecules under TLC Conditions

TLC is one of the most efficient methods used for the fractionation of polymers and the analysis of their heterogeneity. The chromatographic behaviour of polymers differs from that of low MW compounds in many ways that can be revealed even in the analysis of narrow-dispersity homopolymers. Unlike low MW compounds, polymers are characterized by physical heterogeneity, i.e. they are a mixture of macromolecules with different degrees of polymerization (polymer homologues). The concept of 'molecular weight' is replaced by the expression 'average MW', which is a statistical average value. In addition to physical heterogeneity characterized by molecular weight distribution (MWD), chromatographic